# Enzymatic Synthesis of Chiral Intermediates for Drug Development

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**Abstract:** Chirality is a key factor in the efficacy of many drug products and thus the production of single enantiomers of drug intermediates has become increasingly important in the pharmaceutical industry. Biocatalysis is now accepted as a one of key methodologies for the preparation of chiral drug intermediates and fine chemicals. The biocatalytic production of several key intermediates in the synthesis of antihypertensive, anticholesterol, anti-Alzheimer's, β3-receptor agonist, HIV-protease inhibitor, and other pharmaceuticals is described. These includes (1) the synthesis of L-6-hydroxynorleucine from racemic 6-hydroxynorleucine, (2) the enzymatic synthesis of (*S*)-allysine ethylene acetal by reductive deamination using phenylalanine dehydrogenase, (3) the synthesis of [4*S*-(4a,7a,10ab)]-1-octahydro-5-oxo-4-{[(phenylmethoxy)carbonyl]-amino}-7H-pyrido-[2,1-*b*][1,3]thiazepine-7-carboxylic acid (BMS-199541–01) by enzymatic oxidation process using L-lysine-ε-aminotransferase, (4) the enzymatic synthesis of the lactol [3a*S*-(3aα,4α,7α,7aα)]-hexahydro-4,7-epoxyisobenzofuran-1(3*H*)-ol and corresponding lactone, (5) the microbial synthesis of (3*R*-*cis*)-1,3,4,5-tetrahydro-3-hydroxy-4-(4-methoxyphenyl)-6-(trifluoromethyl)-2*H*-1-benzazepin-2-one, (6) the microbial oxygenation of 6-cyano-2,2-dimethyl-2*H*-1-benzopyran to the corresponding chiral epoxide and (+)-*trans* diol, (7) the enantioselective microbial reductions of *N*-[4-(2-chloroacetyl)phenyl]methanesulfonamide and (4-benzyloxy-3-methanesulfonylamino)-2'-bromoacetophenone to the corresponding (*R*)-alcohols, (8) the enzymatic resolution of racemic α-methyl phenylalanine amides by amidase, (9) the enantioselective hydrolysis of diethyl methyl-(4-methoxyphenyl)-propanedioate by lipase PS-30, (10) the enantioselective microbial reduction of methyl 4-chloro-3-oxobutanoate, (11) the enzymatic synthesis of ethyl (3*S*,5*R*)-dihydroxy-6-(benzyloxy)hexanoate, (12) the enantioselective hydrolysis of racemic epoxide 1-{2',3'-dihydrobenzo[*b*]furan-4'-yl}-1,2-oxirane by epoxide hydrolase, (13) the biocatalytic dynamic kinetic resolution of *R*,*S*-1-{2',3'-dihydrobenzo[*b*]furan-4'-yl}-ethane-1,2-diol, and (13) the diastereoselective microbial reduction of (1*S*)-[3-chloro-2-oxo-1-(phenylmethyl)propyl]carbamic acid 1,1-dimethylethyl ester.



**Keywords:** anti-Alzheimer drugs; antihypertensive; anticholesterol; biocatalysis; calcium channel blockers; chiral drug intermediates; HIV-protease inhibitors; potassium channel openers; β3-receptor agonists

## 1 Introduction

Chirality is a key factor in the efficacy of many drug products and agrochemicals, and thus the production of single enantiomers of chiral intermediates has become increasingly important in the pharmaceutical industry.[1] Single enantiomers can be produced by chemical or chemo-enzymatic synthesis. The advantages of biocatalysis over chemical synthesis are that enzyme-catalyzed reactions are often highly stereoselective and regioselective. They can be carried out at ambient temperature and atmospheric pressure, thus avoiding the use of more extreme conditions, which could cause problems with isomerization, ra-

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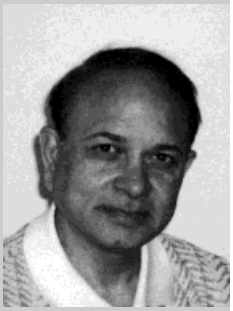

cemization, epimerization, and rearrangement. Microbial cells and enzymes derived therefrom can be immobilized and reused for many cycles. In addition, enzymes can be overexpressed to make biocatalytic processes economically efficient, and enzymes with modified activity can be tailor-made. The preparation of thermostable and pH-stable enzymes produced by random and site-directed mutagenesis has led to the production of novel biocatalysts. A number of review articles[2–16] have been published on the use of enzymes in organic synthesis. This review provides examples of the use of enzymes for the synthesis of single enantiomers of key intermediates for drugs in development at Bristol–Myers Squibb.

## 2 Antihypertensive Drugs

Omapatrilat (**1**) (Figure 1) is an antihypertensive drug which acts by inhibiting angiotensin-converting enzyme (ACE) and neutral endopeptidase.[17] Effective inhibitors of ACE have been used not only in the treatment of hypertension but also in the clinical management of congestive heart failure.[18] Neutral endopeptidase (NEP), like ACE, is a zinc metalloprotease and is highly efficient in degrading atrial natriuretic peptide (ANP), a 28-amino acid peptide secreted by the heart in response to atrial distention. By interaction with its receptor, ANP promotes the generation of cGMP via guanylate cyclase activation, thus resulting in vasodilatation, natriuresis, diuresis, and inhibition of aldosterone.[19] Therefore, simultaneous potentiation of ANP via NEP inhibition and attenuation of AII via ACE inhibition should lead to complementary effects in the management of hypertension and congestive heart failure.[20,21]

The enzymatic and/or microbial synthesis of single enantiomers of three key intermediates in three different routes to Omapatrilat (**1**) were developed as described below.

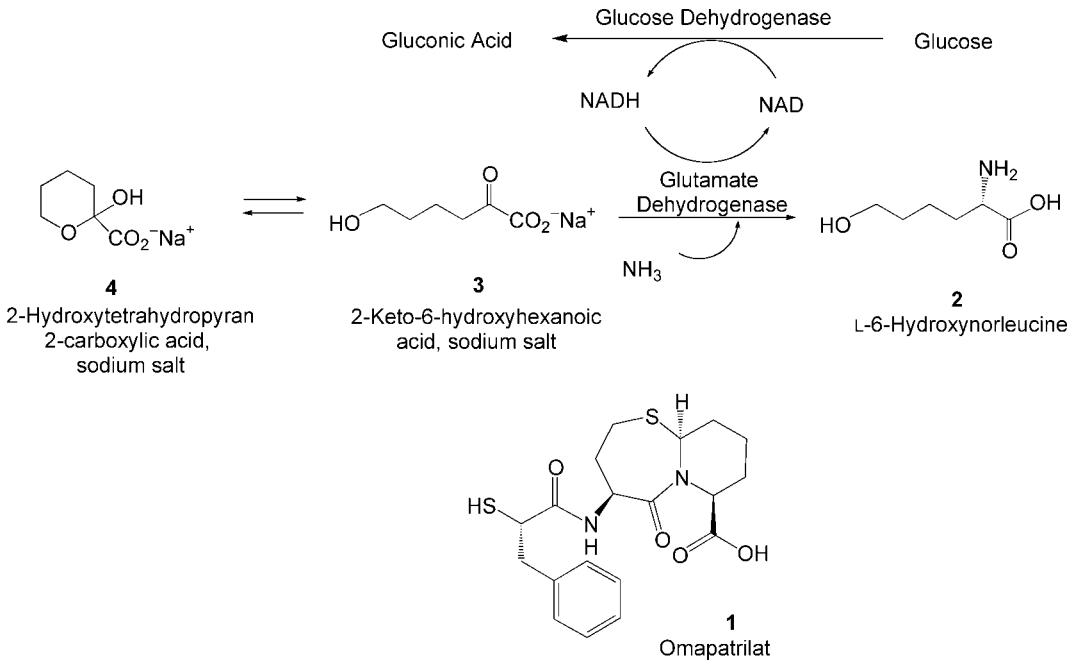


**Figure 1.** Enzymatic synthesis of chiral synthons for an antihypertensive drug: reductive amination of sodium 2-keto-6-hydroxyhexanoic acid (**3**) to L-6-hydroxynorleucine (**2**) by glutamate dehydrogenase. Structure of Omapatrilat (**1**).

### 2.1 Enzymatic Synthesis of L-6-Hydroxynorleucine (2)

L-6-Hydroxynorleucine (**2**) (Figure 1) is a key intermediate useful in the synthesis of Omapatrilat as well as C-7 substituted azepinones which are potential intermediates for other antihypertensive metalloprotease inhibitors.[17,22] Reductive amination of keto-acids using amino acid dehydrogenases has long been known to be a useful method for the synthesis of natural and unnatural amino acids.[23–25] We have developed the synthesis and complete conversion of 2-keto-6-hydroxyhexanoic acid (**3**) to L-6-hydroxynorleucine (**2**) (Figure 1) by reductive amination using phenylalanine dehydrogenase [PDH] from *Sporosarcina* sp. or by beef liver glutamate dehydrogenase.[26] Beef liver glutamate dehydrogenase was used for preparative reactions at 100 g/L substrate concentration. As depicted, 2-keto-6-hydroxyhexanoic acid sodium salt (**3**), in equilibrium with 2-hydroxytetrahydropyran-2-carboxylic acid sodium salt (**4**) was converted to L-6-hydroxynorleucine (**2**). The reaction requires ammonia and NADH. The $NAD^+$ produced during the reaction was recycled to NADH by the oxidation of glucose to gluconic acid using glucose dehydrogenase from *Bacillus megaterium*. The reaction was completed in about 3 h with reaction yields of 92% and enantiomeric excesses (ee) of >99% for L-6-hydroxynorleucine (**2**).

The chemical synthesis and isolation of 2-keto-6-hydroxyhexanoic acid (**3**) required several steps. In a second, more convenient process (Figure 2), the ketoacid was prepared by treatment of racemic 6-hydroxynorleucine (**5**) [produced by hydrolysis of 5-(4-hydroxybutyl)hydantoin (**6**)] with D-amino acid oxidase and catalase. After the ee of the remaining L-6-hydroxynorleucine had risen to >99%, the reductive amination procedure was used to convert the mixture containing 2-keto-6-hydroxyhexanoic acid (**3**) entirely to L-6-hydroxynorleucine (**2**) with yields of 97% and ee's of 98% from racemic 6-hydroxynorleucine at 100 g/L. Sigma porcine kidney D-amino acid oxidase and beef liver catalase or *T. variabilis* whole cells (source of both oxidase and catalase) were used successfully for this transformation. The L-6-hydroxynorleucine (**2**) prepared by the enzymatic process was converted to Omapatrilat as described previously.[17]

### 2.2 Enzymatic Synthesis of Allysine Ethylene Acetal (7)

(*S*)-2-Amino-5-(1,3-dioxolan-2-yl)-pentanoic acid [(*S*)-allysine ethylene acetal, **7**] is one of three build-

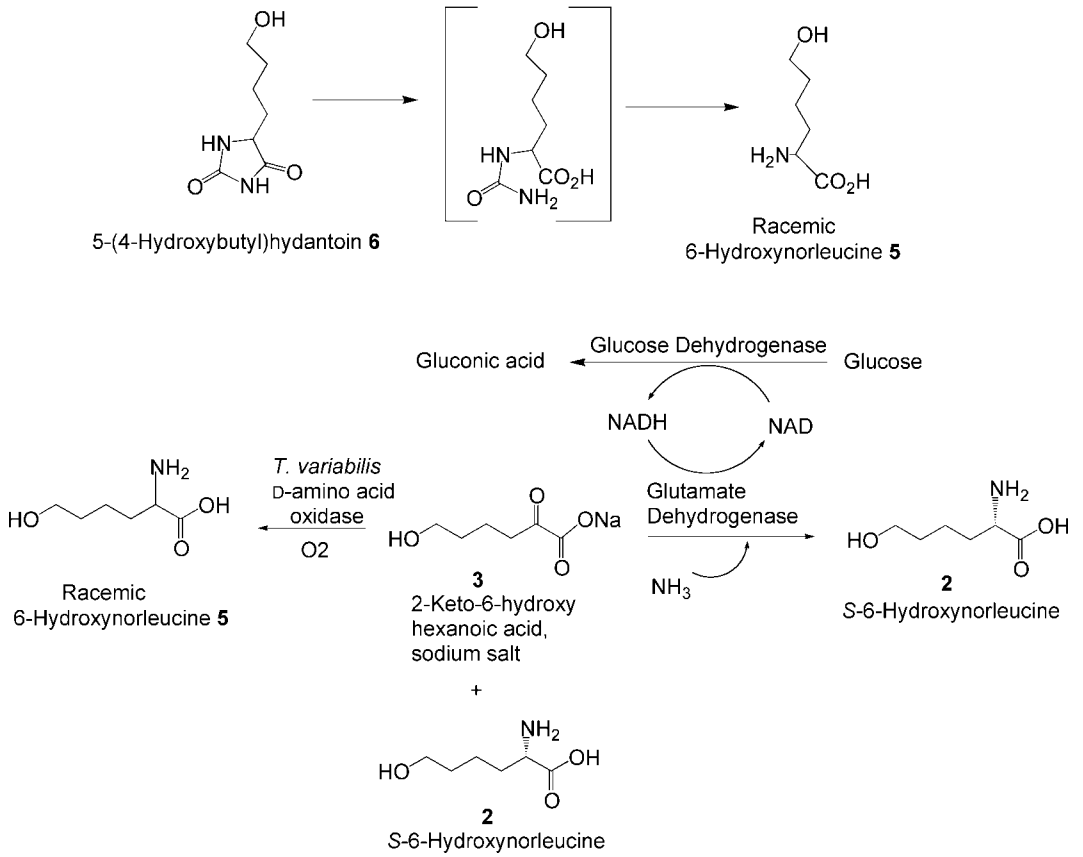


**Figure 2.** Enzymatic synthesis of chiral synthons for an antihypertensive drug: conversion of racemic 6-hydroxynorleucine to L-6-hydroxynorleucine (**2**) by D-amino acid oxidase and glutamate dehydrogenase.

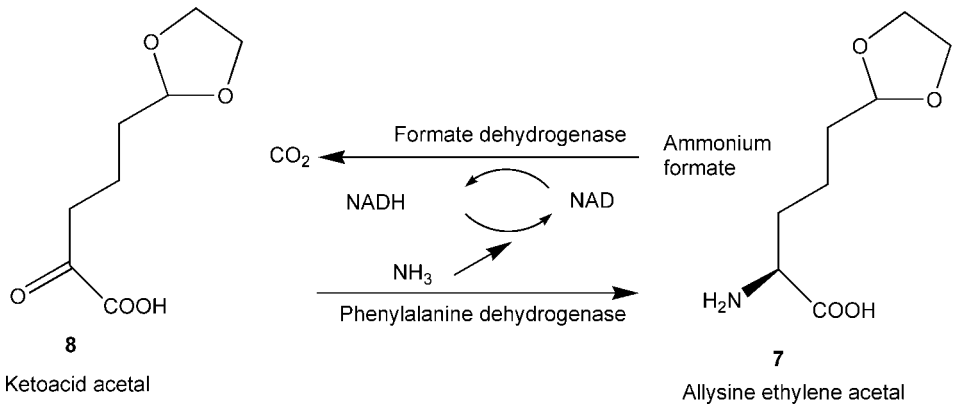


**Figure 3.** Enzymatic synthesis of chiral synthons for an antihypertensive drug: reductive amination of keto acid acetal **8** to amino acid acetal **7** by phenylalanine dehydrogenase. Regeneration of NADH was carried out using formate dehydrogenase.

ing blocks used in an alternative synthesis of Omapatrilat.[17] It previously had been prepared via an eight-step chemical synthesis from 3,4-dihydro-2*H*-pyran.[27] An alternate synthesis of **7** was demonstrated by using phenylalanine dehydrogenase (PDH) from *Thermoactinomyces intermedius* (Figure 3). The reaction required ammonia and NADH; the $NAD^+$ produced during the reaction was recycled to NADH by the oxidation of formate to $CO_2$ using formate dehydrogenase (FDH). An initial process was developed using heat-dried cells of *T. intermedius* ATCC 33205 as a source of PDH and heat-dried cells of methanol-grown *Candida boidinii* as a source of FDH. An improved, second generation process using PDH from *T. intermedius* expressed in *E. coli* BL21(DE3) (pPDH155K) (SC16144) in combination with *C. boidinii* as a source of FDH and a third generation process using methanol-grown *Pichia pastoris* containing endogenous FDH and recombinant protein expressing *T. intermedius* PDH were also developed.[28]

PDH activities and fermentor productivities in cells recovered from fermentations of various cultures are shown in Table 1. *T. intermedius* gave useful activity on a small scale (15 L) but lysed soon after the end of the growth period, making recovery of activity difficult or impossible on a large scale (4000 L). This problem was solved by cloning and expressing the *T. intermedius* PDH in *Escherichia coli*, inducible by β-D-isopropylthiogalactoside (IPTG). Fermentation of *T. intermedius* yielded 184 units of PDH activity per liter of whole broth in 6 hours. In contrast, *E. coli* BL21 (DE3) (pPDH155K) produced over 19,000 units per liter of whole broth in about 14 hours.

*C. boidinii*[29] or *P. pastoris*[30] grown on methanol are useful sources of FDH. Expression of *T. intermedius* PDH in *P. pastoris*, inducible by methanol, allowed us to obtain both enzymes from a single fermentation. Formate dehydrogenase activity/g wet cells in *P. pastoris* was 2.7-fold greater than for *C. boidinii* and fermentor productivity was increased by 8.7-fold compared to *C. boidinii*. Fermentor productivity for PDH in *P. pastoris* was about 28% of the *E. coli* productivity.

Reductive amination reactions were carried out at pH 8.0. A procedure using heat-dried cells of *E. coli* containing cloned PDH and heat-dried *C. boidinii* was scaled up (Table 2). A total of 197 kg of **7** was produced in three 1600-L batches using a 5% concentration of substrate **8** with an average yield of 91 M % and ee >98%.

A third generation procedure, using dried recombinant *P. pastoris* containing *T. intermedius* PDH inducible with methanol and endogenous FDH induced when *P. pastoris* was grown in medium containing methanol, allowed both enzymes to be produced during a single fermentation. The *P. pastoris* reaction procedure had the following modifications over the *E. coli*/*C. boidinii* procedure: the concentration of substrate was increased to 100 g/L, 1/4 the amount of NAD was used, and dithiothreitol was omitted.

**Table 1.** Activities and productivities of phenylalanine dehydrogenase and formate dehydrogenase.

| Enzyme | Strain | Specific activity (U/g wet cells) | Volumetric activity (U/L of broth) | Productivity (U/L/week) |
|---|---|---|---|---|
| Phenylalanine dehydrogenase | *Thermoactinomyces intermedius* | 510 | 185 | 900 |
| | *Escherichia coli* | 10,000 | 24,000 | 94,000 |
| | *Pichia pastoris* | ND | 14,500 | 25,000 |
| Formate dehydrogenase | *Candida boidinii* | 9 | 120 | 350 |
| | *Pichia pastoris* | 26 | 1950 | 3200 |

**Table 2.** Preparative bioconversion of keto acid **8** to L-amino acid **7**.[a]

| Phenylalanine dehydrogenase source | Formate dehydrogenase source | Ketoacid **8** input (kg) | Amino acid **7** output (kg) | Reaction yield of **7** (M %) | ee of amino acid **7** (%) |
|---|---|---|---|---|---|
| *Escherichia coli* | *Candida boidinii* | 80.17 | 62.4 | 92 | >99 |
| *Escherichia coli* | *Candida boidinii* | 79.96 | 66.75 | 96 | >99 |
| *Escherichia coli* | *Candida boidinii* | 89.6 | 67.61 | 86 | >99 |
| *Pichia pastoris* | *Pichia pastoris* | 18.05 | 15.51 | 97.5 | >99 |

[a] Reaction mixtures (1600 L) contained 19.49 g/L ammonium formate, 50 g/L substrate **7**, 0.35 g/L NAD, 1.66 kU/L formate dehydrogenase, 0.27 kU/L phenylalanine dehydrogenase. Reactions were carried out at pH 8.0, 40 °C and 80 rpm for 18 hours.

The procedure with *P. pastoris* was also scaled up to produce 15.5 kg of **7** with 97 M % yield and ee >98% (Table 2) in a 180-L batch using a 10% concentration of ketoacid **8**.

Polyethylene glycol-35,000, NADH, PDH, and FDH were also used in a membrane reactor (10,000 mol-wt. cutoff membrane) to retain and reuse enzymes and cofactor. Preliminary results demonstrated the production of compound **7** from compound **8** at a rate of 1 g/10 mL/day for about 14 days.[31]

The (*S*)-allysine ethylene acetal **7** produced by the enzymatic process was converted to dimethyl acetal methyl ester **9** and coupled to the *N*-protected amino acid **10** to give the dipeptide dimer **11**. This compound was treated with dithiothreitol (DTT) and sodium methoxide in methanol to give the corresponding monomeric mercapto compound which was cyclized by treatment with methanesulfonic acid to give the bicyclic thiazapinone **12** (Figure 4) which was finally converted to Omapatrilat (**1**).[32]

### 2.3 Enzymatic Synthesis of [4*S*-(4a,7a,10ab)]-1-Octahydro-5-oxo-4-{[(phenylmethoxy)carbonyl]- amino}-7*H*-pyrido[2,1-*b*][1,3]thiazepine-7-carboxylic Acid

[4*S*-(4a,7a,10ab)]-1-Octahydro-5-oxo-4-{[(phenylmethoxy)carbonyl]amino}-7*H*-pyrido-[2,1-*b*][1,3]-thiazepine-7-carboxylic acid [BMS-199541–01] (**12**) is a key intermediate in the alternate synthesis of Omapatrilat (**1**). Our goal was to prepare the compound by a simpler route using an intermediate derived from L-lysine as a readily available starting material. An enzymatic process was developed for the oxidation of the ε-amino group of lysine in the thiol **13** generated *in situ* from dipeptide dimer (disulfide) $N^2$-{*N*-[[(phe-

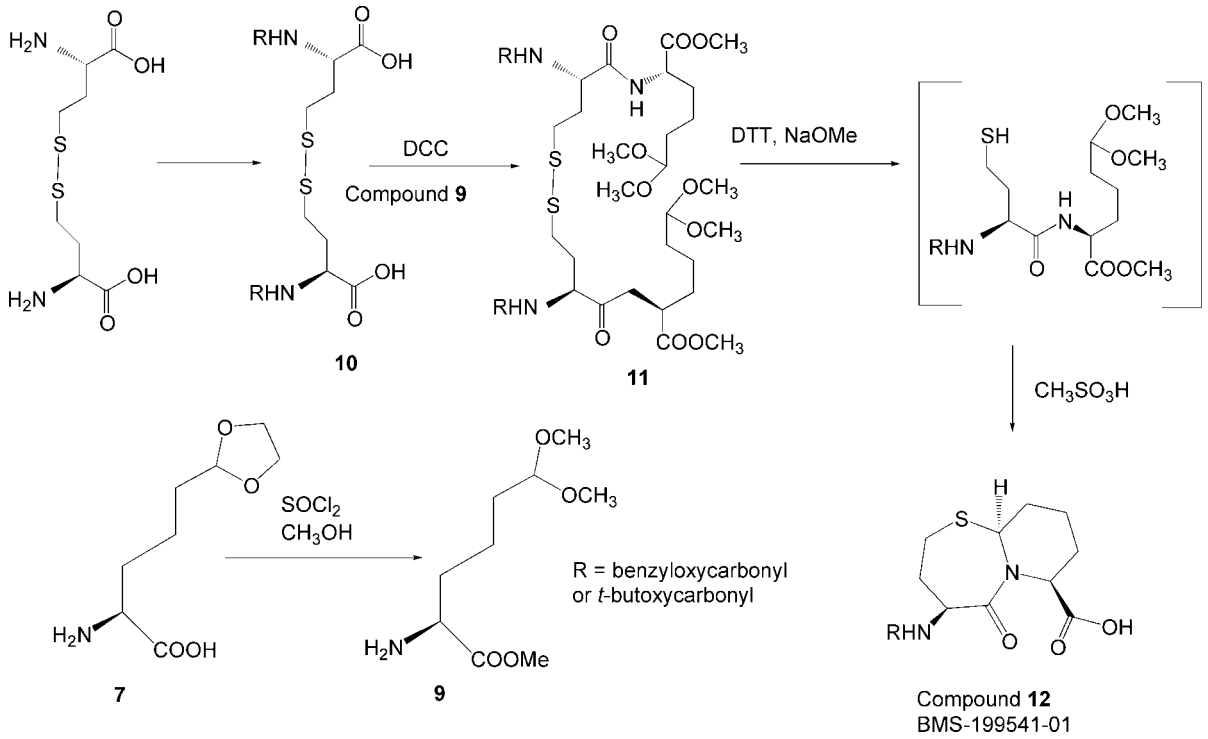


**Figure 4.** Chemical conversion of amino acid acetal **7** to compound **12** (BMS-199541–01).

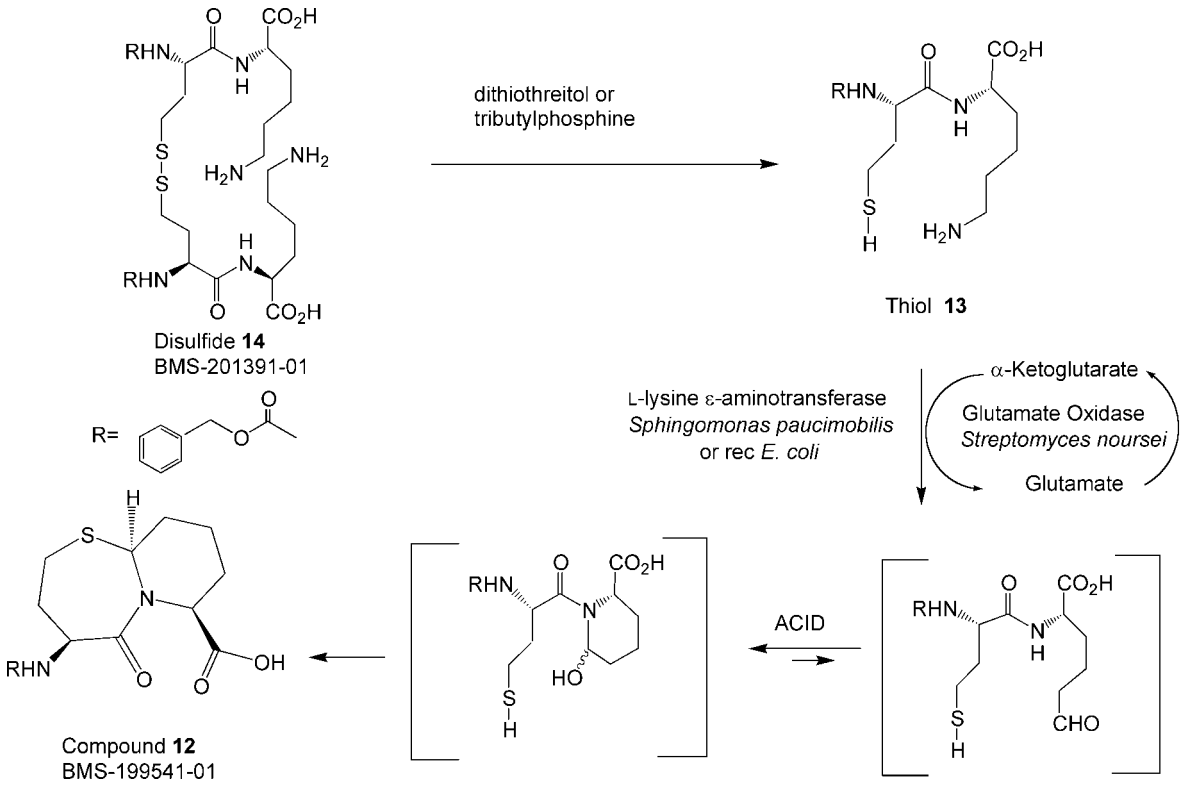


**Figure 5.** Enzymatic synthesis of chiral synthons for an antihypertensive drug: conversion of compound **14** (BMS-201391–01) to compound **12** (BMS-199541–01) by L-lysine ε-aminotransferase.

nylmethoxy)carbonyl]-L-homocysteinyl]-L-lysine} 1,1-disulfide (BMS-201391–01) (**14**) to produce BMS-199541–01 **12** (Figure 5) using L-lysine ε-aminotransferase (LAT) from *Sphingomonas paucimobilis* SC 16113.[33] This enzyme was overexpressed in *E. coli* and a biotransformation process was developed using the recombinant enzyme. The aminotransferase reaction required α-ketoglutarate as the amine acceptor. Glutamate formed during this reaction was recycled back to α-ketoglutarate by glutamate oxidase (GOX) from *Streptomyces noursei* SC 6007.

A selective culture technique was used to isolate microorganisms able to utilize *N*-α-Cbz-L-lysine as the sole source of nitrogen. Using this technique, eight different types of microbial colonies were isolated. Cultures were grown in shake flasks and cell extracts prepared from cell suspensions were evaluated for oxidation of the ε-amino group of L-lysine in the substrate **13** generated from BMS-201391–01 **14**. Product BMS-199541–01 **12** formation was observed with four cultures. One of the cultures, Z-2, later identified as *S. paucimobilis* SC 16113, exhibited higher activity (0.35 mg/mL of product formed) and was used for further studies.

*S. paucimobilis* SC 16113 was grown in a 700-L fermentor containing 500 L of medium. A preparative batch for biotransformation of BMS-201391–01 to BMS-199541–01 using 2 L of cell extract of *S. paucimobilis* SC 16113 was prepared. The substrate was used at a concentration of 1.5 g/L. A reaction yield of only 10% (0.3 g of BMS-199541–01 **12**) was obtained after 1.75 hours. The product was isolated and identified by $^{1}$H NMR, $^{13}$C NMR, and mass analysis. The low reaction yield was due to the hydrolysis of the substrate BMS-201391–01 **14** by proteases present in cell extracts of *S. paucimobilis* SC 16113.

Due to the low activity of LAT in *S. paucimobilis* SC 16113 and to minimize dipeptide hydrolysis, we decided to purify the enzyme, determine its sequence, and overexpress the protein in a suitable host. The enzyme was purified 254-fold to homogeneity resulting in a specific activity (mg product formed/h/g of protein) of 36,600. The molecular weight of the enzyme was 81,000 daltons and the subunit size was 40,000 daltons, indicating that the LAT is a dimeric protein. The N-terminal and internal peptide sequence (generated by Lys-peptidase treatment) of purified LAT were determined to prepare oligonucleotides probes from sequence information for cloning of LAT protein. The LAT was cloned and overexpressed in *E. coli* strain GI724 (pAL781-LAT).

Screening of microbial cultures led to the identification of *S. noursei* SC 6007 as a source of extracellular GOX. *S. noursei* SC 6007 was grown in 380-L fermentors. GOX activity correlated with growth of the

culture in a fermentor and reached 0.75 units/mL at harvest. Starting from the extracellular filtrate recovered after removal of cells from the fermentation broth, the GOX was purified 260-fold to homogeneity with a specific activity (units/mg of protein) of 54. The molecular weight of the enzyme was 125,000 daltons and the subunit size was 60,000 daltons, indicating that the GOX is a dimeric protein. The amino terminal and internal peptide sequence of the purified enzyme were determined to allow for the synthesis of oligonucleotide probes for cloning and overexpression of the enzyme. Attempts to express the *S. noursei* SC 6007 GOX using standard *E. coli* vectors and strains were unsuccessful. As an alternative, the SC 6007 GOX was expressed in *Streptomyces lividans*. About 0.4 units/mL of activity was detected from the *S. lividans* culture indicating that the enzyme was expressed at a low level.

Biotransformation of BMS-201391–01 **14** to BMS-199541–01 **12** was carried out using LAT from *Escherichia coli* GI724 (pal781-LAT) in the presence of α-ketoglutarate and dithiothreitol (DTT), required to reduce the disulfide **14** to a thiol **13**. Glutamate produced during the reaction was recycled to α-ketoglutarate by partially purified GOX from *S. noursei* SC 6007. Four different batches were carried out. Reaction yields of 65 – 67 M % were obtained (Table 3). To reduce the cost of producing two enzymes, the transamination reactions were carried out in the absence of GOX and higher levels of α-ketoglutarate. The reaction yield in the absence of GOX averaged only about 33 – 35 M %. However, the reaction yield increased to 70 M %, by increasing the α-ketoglutarate to 40 mg/mL of (10-fold increase in concentration) and conducting the reaction at 40 °C, equivalent to that in the presence of GOX. Phenylacetyl- or phenoxyacetyl-protected analogues of BMS-201391–01 (Figure 5) also served as substrates for LAT, giving reaction yields of 70 M % for the corresponding BMS-199541–01 analogues. In the enzymatic reaction to convert BMS-201391–01 to BMS-199541–01, we used DTT to cleave the disulfide bond of the compound **14** to produce the compound **13**, which was the substrate for the LAT. It was observed that tributylphosphine (an inexpensive reagent) was as effective as DTT for this conversion. To terminate the LAT reaction and to cyclize the product of LAT oxidation during conversion of compound **14** to compound **12**, 10% v/v trichloroacetic acid (TCA) was used. It was also observed that the much cheaper compound methanesulfonic acid is equally effective as TCA, giving a 70 M % yield of compound **12**.

## 3 Thromboxane $A_2$ Antagonists

Thromboxane $A_2$ (TxA2) is an exceptionally potent vasoconstrictor substance produced by the metabolism of arachidonic acid in blood platelets and other tissues. Together with its potent anti-aggregatory and vasodilator activities, TxA2 plays an important role in the maintenance of vascular homeostasis, and contributes to the pathogenesis of a variety of vascular disorders. Approaches towards limiting the effect of TxA2 have focused on either inhibiting its synthesis or blocking its action at its receptor sites by means of an antagonist.[34,35] The lactol [3a*S*-(3aα,4α,7α,7aα)]-hexahydro-4,7-epoxyisobenzofuran-1(3*H*)-ol (**15**) or the corresponding chiral lactone **16** (Figure 6) are key intermediates in the total synthesis of {1*S*-[1α,2α(*Z*),3α,4α]}-7-{3-[(2-heptanoylaminoacetylamino)methyl]-7-oxabicyclo[2.2.1]hept-2-yl}-hept-5-enoic acid (**17**), a new cardiovascular agent useful in the treatment of thrombotic disease.[36,37]

We have[38] described the stereoselective oxidation of (*exo,exo*)-7-oxabicyclo[2.2.1]heptane-2,3-dimethanol (**18**) to the corresponding chiral lactol **15** and lactone **16** [Figure 6, path A] by cell-suspensions (10% w/v, wet cells) of *Nocardia globerula* ATCC 21505 and *Rhodococcus* sp. ATCC 15592. Lactone **16** was obtained in 70 M % yield with an ee of 96% after 96 hours at 5 g/L substrate concentration using cell suspensions of *N. globerula* ATCC 21505. An overall reaction yield of 46 M % (lactol and lactone combined) and ee's of 96.7% and 98.4% were obtained for lactol **15** and lactone **16**, respectively, using cell suspensions of *Rhodococcus* sp. ATCC 15592; substrate **18** was used at a 5 g/L concentration.

The asymmetric hydrolysis of the diacetate of (*exo,exo*)-7-oxabicyclo[2.2.1]heptane-2,3-dimethanol **19** to the corresponding (*S*)-monoacetate ester **20** (Figure 6, path B) has been demonstrated with li-

**Table 3.** Biotransformation of BMS-201391–01 to BMS-199541–01 by L-lysine ε-aminotransferase from *Escherichia coli* GI724 (pal781-LAT).[a]

| BMS-201391–01 input (g) | BMS-201391–01 remaining (g) | BMS-199541–01 (g) | BMS-199541–01 (M % yield) |
|---|---|---|---|
| 3 | 0.83 | 1.9 | 66.5 |
| 5 | 1.35 | 2.92 | 65 |
| 12 | 4.3 | 7.5 | 70 |
| 22 | 4.7 | 14.4 | 67 |

[a] Reactions were carried out using cell extracts of *Escherichia coli* GI724 (pal781-LAT) in the presence of dithiothreitol and partially purified glutamate oxidase from *Streptomyces noursei* SC 6007.

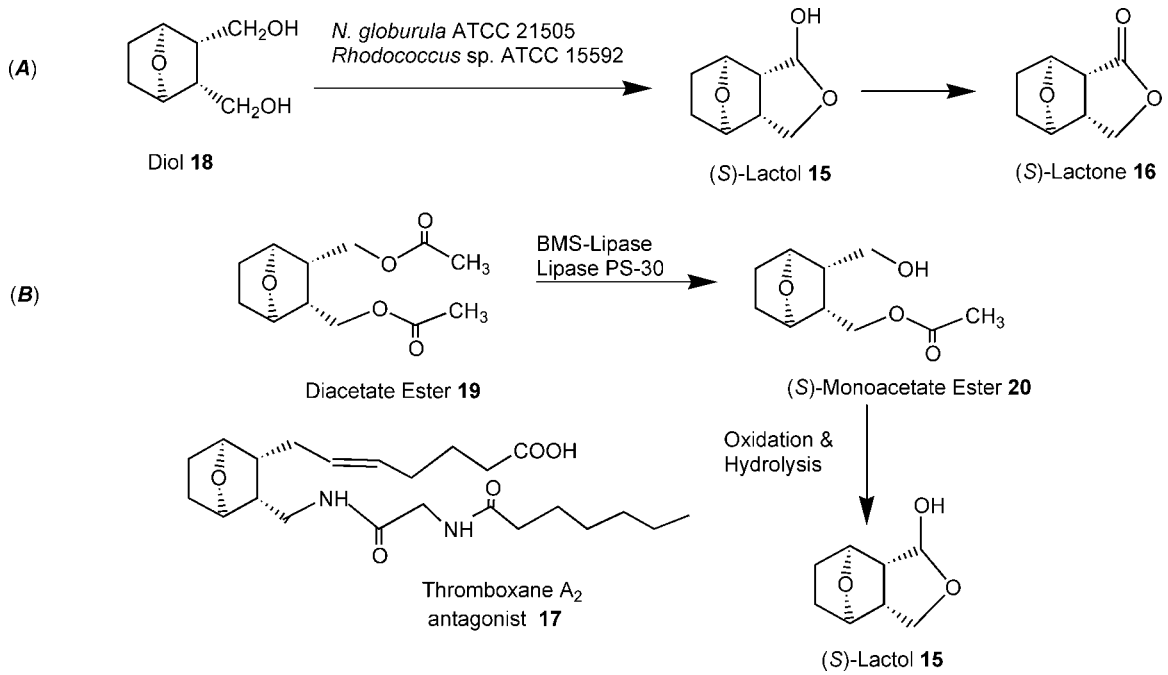


**Figure 6.** Synthesis of chiral synthons for the thromboxane $A_2$ antagonist **17**: (A) stereoselective oxidation of diol **18** to lactol **15** and lactone **16**; (B) asymmetric enzymatic hydrolysis of diacetate ester **19** to the (*S*)-monoacetate ester **20**.

pases.[39] Lipase PS-30 from *P. cepacia* was the most effective in the asymmetric hydrolysis to the desired (*S*)-monoacetate ester. A reaction yield of 75 M % and ee of >99% was obtained when the reaction was conducted in a biphasic system with 10% toluene at 5 g/L of the substrate. Lipase PS-30 was immobilized on Accurel polypropylene (PP) and the immobilized enzyme was reused (5 cycles) without loss of enzyme activity, productivity or ee of product **20**. The reaction process was scaled-up to 80 L (400 g of substrate) and the (*S*)-monoacetate ester **20** was isolated in 80 M % yield with 99.3% ee. The (*S*)-monoacetate ester **20** was oxidized to its corresponding aldehyde, which was hydrolyzed to give the lactol **15**, which was used in the chemo-enzymatic synthesis of thromboxane $A_2$ antagonist **17**.

## 4 Calcium Channel Blocking Drugs

Diltiazem (**21**), a benzothiazepinone calcium channel blocking agent that inhibits influx of extracellular calcium through L-type voltage-operated calcium channels, has been widely used clinically in the treatment of hypertension and angina.[40] Since diltiazem has a relatively short duration of action,[41] an 8-chloro derivative has recently been introduced into the clinic as a more potent analogue.[42] Lack of extended duration of action and little information on structure-activity relationships in this class of compounds led Floyd et al.[43] and Das et al.[44] to prepare isosteric 1-benzazepin-2-ones; this led to the identification of (*cis*)-3-(acetoxy)-1-[2-(dimethylamino) ethyl]-1,3,4,5-tetrahydro-4-(4-methoxyphenyl)-6-trifluoromethyl)-2*H*-1-benzazepin-2-one (**22**) as a longer-acting and more potent antihypertensive agent. A key intermediate in the synthesis of this compound was (3*R-cis*)-1,3,4,5-tetrahydro-3-hydroxy-4-(4-methoxyphenyl)-6-(trifluoromethyl)-2*H*-1-benzazepin-2-one (**23**). A stereoselective microbial process (Figure 7) was developed for the reduction of 4,5-dihydro-4-(4-methoxyphenyl)-6-(trifluoromethyl)-1*H*-1-benzazepine-2,3-dione (**24**), which exists predominantly in the achiral enol form in rapid equilibrium with the two enantiomeric keto forms. Reduction of **24** could give rise to formation of four possible alcohol stereoisomers. Remarkably, conditions were found under which only the single alcohol isomer **23** was obtained by microbial reduction. Among various cultures evaluated, microorganisms from the genera *Nocardia, Rhodococcus*, *Corynebacterium,* and *Arthobacter* reduced compound **24** to compound **23** with 60 – 70% conversion yield at 1 g/L substrate concentration. The most effective culture, *Nocardia salmonicolor* SC 6310, catalyzed the bioconversion of **24** to **23** in 96% reaction yield with 99.8% ee at 2 g/L substrate concentration. A preparative-scale fermentation process for growth of *N. salmonicolor* and a bioreduction process using cell suspensions of the organism were demonstrated.[45]

## 5 Potassium Channel Openers

The study of potassium (K) channel biochemistry, physiology, and medicinal chemistry has flourished,

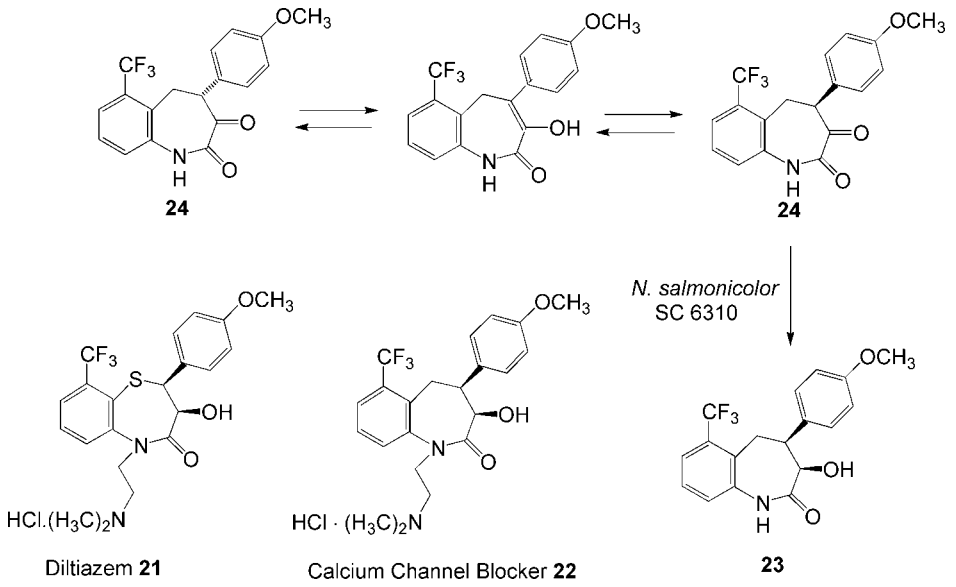


**Figure 7.** Microbial synthesis of chiral synthons for the calcium channel blocker **22**: stereoselective reduction of 4,5-dihydro-4-(4-methoxyphenyl)-6-(trifluoromethyl)-1*H*-benzazepine-2,3-dione (**24**).

with numerous papers and reviews having been published in recent years.[46,47] It has long been known that K channels play a major role in neuronal excitability and a critical role in the basic electrical and mechanical function of a wide variety of tissues, including smooth muscle and cardiac muscle.[48] A new class of highly specific pharmacological compounds which either open or block K channels has been developed.[49,50] Recently, the synthesis and anti-hypertensive activity of a series of novel K-channel openers[51–54] based on monosubstituted *trans*-4-amino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-3-ol (**25**) have been established. Chiral epoxide **26** and diol **27** are potential intermediates for the synthesis of **25**. The stereoselective microbial oxygenation of 2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile **28** to the corresponding chiral epoxide **26** and chiral diol **27** (Figure 8) has been demonstrated.[55] Among the microbial cultures evaluated, the two cultures, *Mortierella ramanniana* SC 13840 and *Corynebacterium* sp. SC 13876 gave reaction yields of 67.5 M % and 32 M % and ee's of 96% and 89%, respectively, for (+)-*trans* diol **27**. *Corynebacterium* sp. SC 13876 produced the chiral epoxide **26** in 17 M % yield and 88% ee.

A single-stage process (fermentation/epoxidation) for the biotransformation of **28** was developed using *M. ramanniana* SC 13840. In a 25-L fermentor, (+)-*trans* diol **27** was obtained in a 61 M % yield and an ee of 92.5%. In a two-stage process using a cell-suspension (10% w/v, wet cells) of *M. ramanniana* SC 13840, the (+)-*trans* diol **27** was obtained in

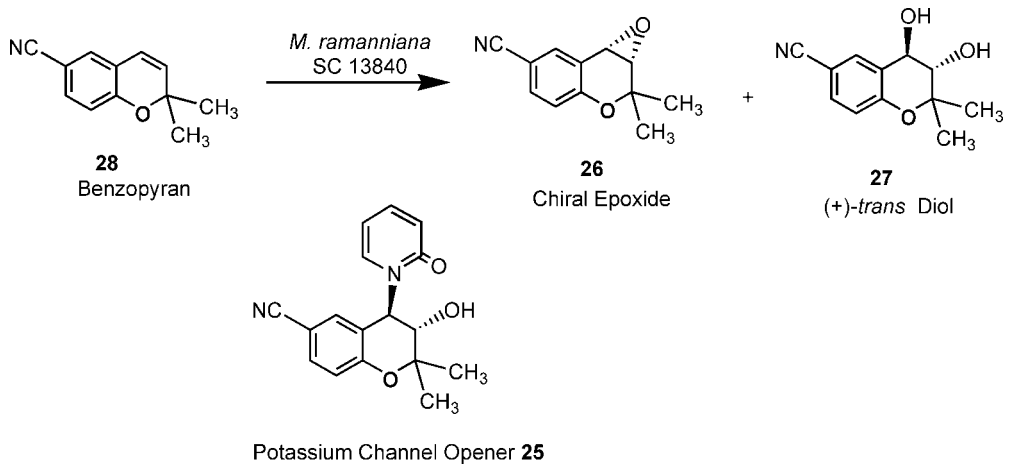


**Figure 8.** Microbial synthesis of chiral synthons for the potassium channel opener **25**: oxygenation of 2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile **28** to the corresponding chiral epoxide **26** and chiral diol **27**.

76 M % yield with an ee of 96%. The reaction was carried out in a 5-L Bioflo fermentor with 2 g/L substrate and 10 g/L glucose concentrations. Glucose was supplied to regenerate the NADH required for this reaction. From the reaction mixture, (+)-*trans* diol **27** was isolated in 65 M % overall yield. An ee of 97% and a chemical purity of 98% were obtained.

In an enzymatic resolution approach, chiral (+)-*trans* diol **27** was prepared by the stereoselective acetylation of racemic diol with lipases from *Candida cylindraceae* and *P. cepacia*. Both enzymes catalyzed the acetylation of the undesired enantiomer of the racemic diol to yield the monoacetylated product and unreacted desired (+)-*trans* diol **27**. A reaction yield of 40% (theoretical maximum yield is 50%) and an ee of >90% were obtained using each lipase.[56]

## 6 Antiarrhythmic Agents

The biological activity of a series of phenethanolamines bearing alkylsulfonamido groups have been reported.[57] Within this series, some compounds possessed adrenergic activity while others demonstrated antiadrenergic actions. (*R*)-(+)-Sotalol (**29**) is a β-blocker[58,59] that, unlike other β-blockers, has antiarrhythmic properties and no other peripheral action. The β-adrenergic blocking drugs such as propranolol and sotalol have been separated chemically into the (*S*)- and (*R*)-rotatory optical isomers, and it has been demonstrated that the activity of the (*R*) isomer is 50 times that of the corresponding (*S*) isomer.[60] Chiral alcohol **30** is a key intermediate for the chemical synthesis of (*R*)-(+)-sotalol (**29**). The enantioselective microbial reduction of *N*-[4-(2-chloroacetyl)phenyl]methanesulfonamide (**31**) to the corresponding (*R*)-alcohol **30** (Figure 9) has been demonstrated.[61] Among numbers of microorganisms screened for the transformation of ketone **31** to (*R*)-(+)-alcohol **30**, *Rhodococcus* sp. ATCC 29675, ATCC 21243, *N. salmonicolor* SC 6310, and *Hansenula polymorpha* ATCC 26012 gave the desired alcohol **30** in >90% ee. *H. polymorpha* ATCC 26012 catalyzed the efficient conversion of ketone **31** to (*R*)-(+)-alcohol **30** in 95% reaction yield and >99% ee. Growth of *H. polymorpha* ATCC 26012 culture was carried out in a 380-L fermentor and cells harvested from the fermentor were used to conduct the transformation in a 3-L preparative batch. Cell suspensions (20% wet cells in 3 L of 10 mM potassium phosphate buffer pH 7.0) were supplemented with 12 g of ketone **31** and 225 g of glucose and the reduction reaction was carried out at 25 °C, 200 rpm, pH 7. Complete conversion was obtained in a 20-hour reaction period. Using preparative HPLC, 8.2 g of (*R*)-(+)-alcohol **30** were isolated from the reaction mixture in overall 68% yield with >99% ee.

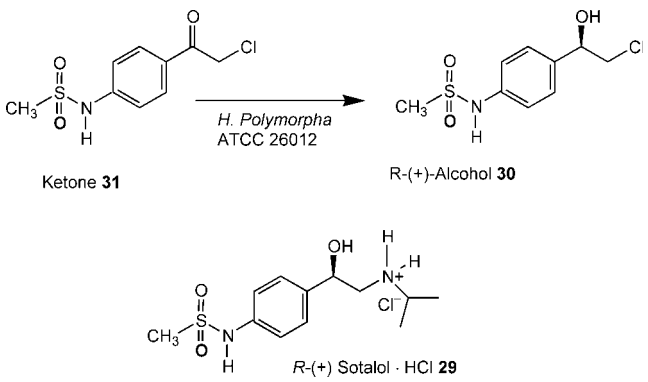


**Figure 9.** Synthesis of chiral intermediates for an antiarrhythmic agent: stereoselective microbial reduction of *N*-[4-(2-chloroacetyl)phenyl]methanesulfonamide **31** to the (*R*)-alcohol **30**.

## 7 β3-Receptor Agonists

β-Adrenoceptors have been classified as β1 and β2.[62] Increased heart rate is the primary consequence of β1-receptor stimulation, while bronchodilation and smooth muscle relaxation are mediated from β2 receptor stimulation. Rat adipocyte lipolysis was initially thought to be a β1-mediated process.[62] However, recent results indicate that the receptor-mediated lipolysis is neither β1 nor β2, but "atypical" receptors, now called β3-adernergic receptors.[63] β3-Adrenergic receptors are found on the cell surface of both white and brown adipocytes and are responsible for lipolysis, thermogenesis, and relaxation of intestinal smooth muscle.[64] Consequently, several research groups are engaged in developing selective β3 agonists for the treatment of gastrointestinal disorders, type II diabetes, and obesity.[65–67] Three different biocatalytic syntheses of chiral intermediates required for the total synthesis of β3-receptor agonists **32** have been investigated.[68]

### 7.1 Microbial Reduction of 4-Benzyloxy-3-methanesulfonylamino-2'-bromoacetophenone

The microbial reduction of 4-benzyloxy-3-methanesulfonylamino-2'-bromoacetophenone (**33**) to the corresponding (*R*)-alcohol **34** was demonstrated[68] using *S. paucimobilis* SC 16113 (Figure 10). The fermentation of *S. paucimobilis* SC 16113 was carried out in a 750-L fermentor. From each fermentation batch, about 60 kg of wet cell paste were collected. Cells harvested from the fermentor were used to conduct the biotransformation in 1-L, 10-L, and 210-L preparative batches under aerobic or anaerobic conditions. The cells were suspended in 80 mM potassium phosphate buffer (pH 6.0) at 20% (wt/vol, wet cells) concentration. Compound **33** (2 g/L) and glu-

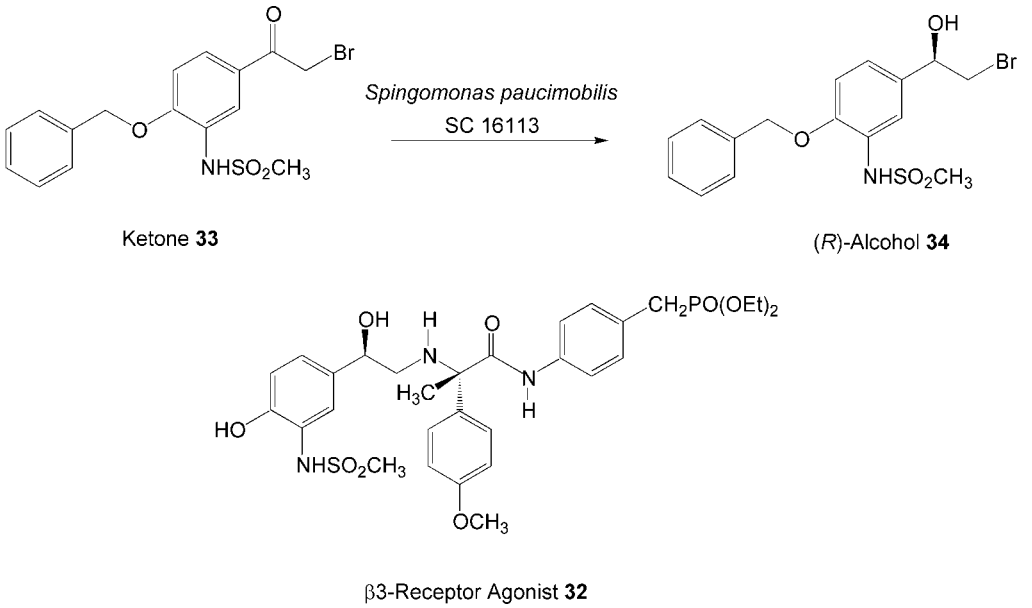


**Figure 10.** Enzymatic synthesis of chiral synthons for the β3-receptor agonist **32**: stereoselective reduction of 4-benzyloxy-3-methanesulfonylamino-2'-bromoacetophenone (**33**) to (*R*)-alcohol **34**.

cose (25 g/L) were added to the fermentor and the reduction reaction was carried out at 37 °C. In some batches, the fermentation broth was concentrated 3-fold by microfilteration and subsequently washed with buffer by diafiltration and used directly in the bioreduction process. In all batches of biotransformation, reaction yields of >85% and ee's of >98% were obtained. The isolation of chiral alcohol **34** from the 200-L preparative batch gave 320 g (80% yield) of product with an ee of 99.5%.

In an alternate process, frozen cells of *S. paucimobilis* SC 16113 were used with XAD-16 resin-adsorbed substrate at 5 g/L and 10 g/L substrate concentrations. In this process, an average reaction yield of 85% and an ee of >99% were obtained for alcohol **34**. At the end of the biotransformation, the reaction mixture was filtered on a 100 mesh (150 μ) stainless steel screen, and the resin retained by the screen was washed with 2 L of water. The product was then desorbed from the resin and crystallized in an overall 75 M % yield and 99.8% ee.

### 7.2 Enzymatic Resolution of Racemic α-Methylphenylalanine Amides

The chiral amino acids **35** and **36** are intermediates for the syntheses of a β3-receptor agonist.[65–67] These are available via the enzymatic resolution of racemic α-methylphenylalanine amide (**37**) and α-methyl-4-methoxyphenylalanine amide (**38**) (Figure 11), respectively, by an amidase from *Mycobacterium neoaurum* ATCC 25795.[68] With 10% wt/vol wet cells the reaction was completed in 75 min with a yield of 48 M % (theoretical maximum 50%) and an ee of 95% for the desired (*S*)-amino acid **35**. Alternatively, freeze-dried cells were suspended in 100 mM potassium phosphate buffer (pH 7.0) at 1% concentration to give complete reaction in 60 min with a yield of 49.5 M % (theoretical maximum 50%) and an ee of 99% for the (*S*)-amino acid **35**.

Freeze-dried cells of *M. neoaurum* ATCC 25795 and partially purified amidase (amidase activity in cell extracts purified 5-fold by diethylaminoethylcellulose column chromatography) were used for the biotransformation of compound **38**. A reaction yield of 49 M % and an enantiomeric excess of 78% were obtained for the desired product **36** using freeze-dried cells. The reaction was completed in 50 hours. Using partially purified amidase, a reaction yield of 49 M % and a higher ee of 94% were obtained after 70 hours reaction time.

### 7.3 Asymmetric Hydrolysis of Racemic Methyl-(4-methoxyphenyl)-propanedioic Acid Diethyl Diester

The (*S*)-monoester **39** is a key intermediate for the syntheses of β3-receptor agonists. The enzymatic asymmetric hydrolysis of diester **40** to the desired acid ester **39** by pig liver esterase[68] has been demonstrated (Figure 12). In various organic solvents the reaction yields and ee of desired acid ester **39** were dependent upon the solvent used. High ee's (>91%) were obtained with methanol, ethanol, and toluene as a cosolvent. Ethanol gave the highest reaction yield (96.7%) and ee (96%) for the desired acid ester **39**.

The effect of temperature and pH were evaluated in a biphasic system using ethanol as a cosolvent. It was observed that the ee of the (*S*)-monoester **39** was in-

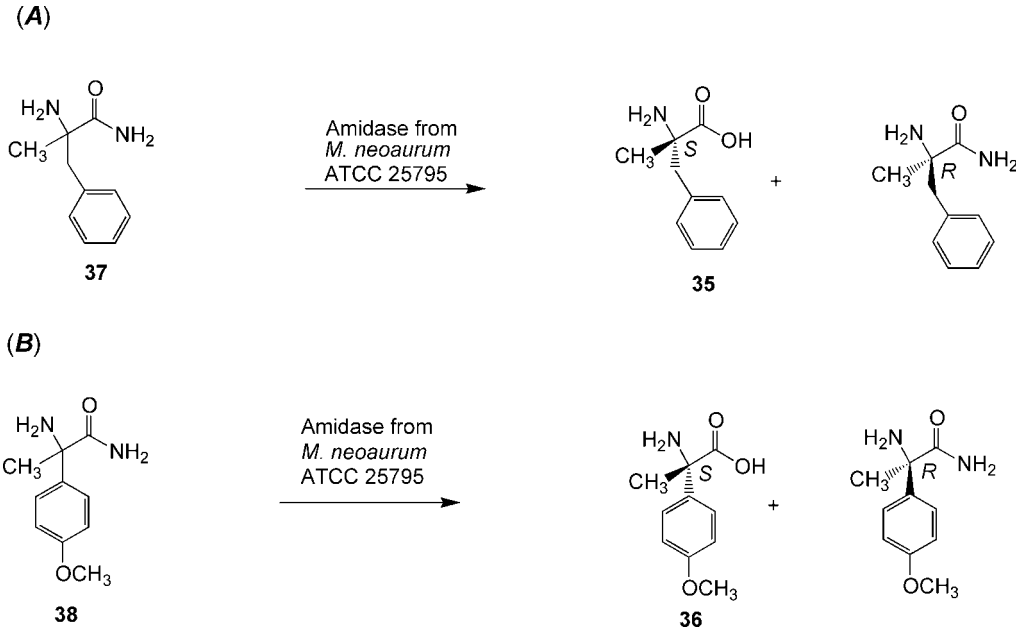


**Figure 11.** Enzymatic synthesis of chiral synthons for the β3-receptor agonist **32**: (A) enantioselective hydrolysis of α-methyl-phenylalanine amide **37** and (B) α-methyl-4-hydroxyphenylalanine amide **38** by amidase.

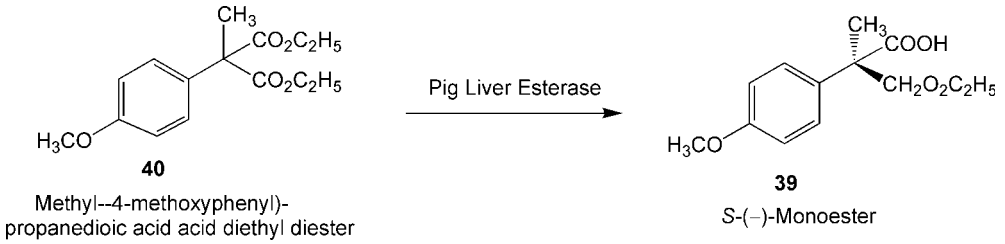


**Figure 12.** Enzymatic synthesis of chiral synthons for the β3-receptor agonist **32**: asymmetric hydrolysis of methyl-(4-methoxyphenyl)-propanedioic acid diethyl diester **40** to the (*S*)-monoester **39**.

creased by decreasing the temperature from 25 °C to 10 °C. A semi-preparative 30-g scale hydrolysis was carried out using 10% ethanol as a cosolvent in a 3-L reaction mixture at 10 °C, 125 rpm agitation, and at pH 7.2 for 11 h. A reaction yield of 96 M % and an ee of 96.9% were obtained. From the reaction mixture, 26 g (86 M % overall yield) of (*S*)-monoester **39** of 96.9% ee were isolated.

## 8 Anticholesterol Drugs

Chiral β-hydroxy esters are versatile synthons.[69–71] The well-known asymmetric reduction of carbonyl compounds using baker's yeast has been reviewed.[72,73] We have described the reduction of 4-chloro-3-oxobutanoic acid methyl ester **41** to (*S*)-(–)-4-chloro-3-hydroxybutanoic acid methyl ester **42** (Figure 13) by cell suspensions of *Geotrichum candidum* SC 5469.[74] Compound (*S*)-(–)-**42** is a key chiral intermediate in the total chemical synthesis of **43**, a cholesterol antagonist which acts by inhibiting hydroxymethylglutaryl-CoA (HMG-CoA) reductase. In the biotransformation process, a reaction yield of 95% and ee of 96% were obtained for (*S*)-(–)-**42** by glucose-, acetate-, or glycerol-grown cells (10% w/v) of *G. candidum* SC 5469. The substrate was used at 10 g/L concentration. The ee of (*S*)-(–)-**42** was increased to 98% by heat-treatment of cell suspensions (55 °C for 30 minutes) prior to conducting the bioreduction. Glucose-grown cells of *G. candidum* SC 5469 have also catalyzed the stereoselective reduction of the ethyl, isopropyl, and *tert*-butyl esters of 4-chloro-3-oxobutanoic acid and the methyl and ethyl esters of 4-bromo-3-oxobutanoic acid. Reaction yields of >85% and ee's of >94% were obtained. The enantioselective $NAD^+$-dependent oxido-reductase was purified 100-fold to homogeneity. The molecular weight of the purified enzyme is 950,000. The purified oxido-reductase was immobilized on Eupergit C and used to catalyze the reduction of **41**. The cofactor $NAD^+$ required for the reduction reaction was regenerated by glucose dehydrogenase. A 90% yield and 98% ee were obtained for (*S*)-(–)-**42**.

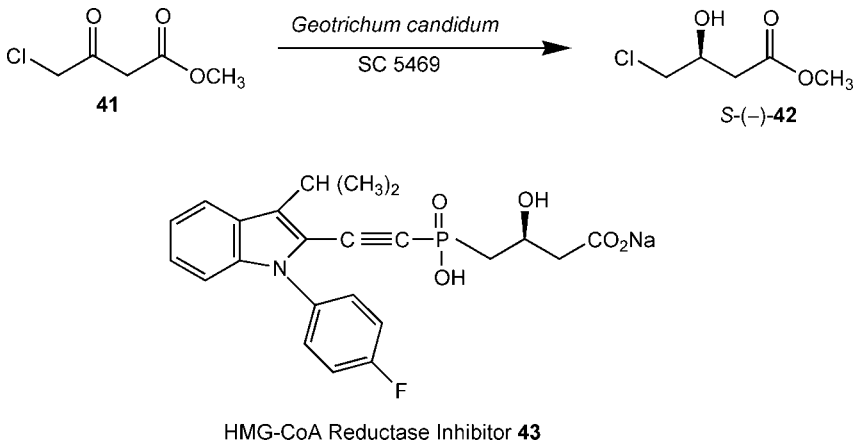


**Figure 13.** Preparation of chiral synthons for the anticholesterol drug **43**: stereoselective microbial reduction of 4-chloro-3-oxobutanoic acid methyl ester **41**.

Many microorganisms and enzymes derived therefrom have been used in the reduction of a single keto group of β-keto or α-keto compounds.[75–78] Patel et al.[79] have demonstrated the stereoselective reduction of a diketone, 3,5-dioxo-6-(benzyloxy)hexanoic acid ethyl ester (**44**), to (3*R*,5*S*)-dihydroxy-6-(benzyloxy)hexanoic acid ethyl ester (**45a**) (Figure 14). The compound **45a** is a key chiral intermediate required for the chemical synthesis of {4-[4a,6ß(E)]}-6-[4,4-bis(4-fluorophenyl)-3-(1-methyl-1*H*-tetrazol-5-yl)-1,3-butadienyl]tetrahydro-4-hydroxy-2*H*-pyren-2-one (**46**), a new anticholesterol drug which acts by inhibition of HMG-CoA reductase.[80] Among various microbial cultures evaluated for the stereoselective reduction of diketone **44**, glycerol-grown cell suspensions of *Acinetobacter calcoaceticus* SC 13876 were shown to give a reaction yield of 85% and ee of 97%. The substrate and cells were used at 2 g/L and 20% (w/v, wet cells) concentration, respectively.

Cell extracts of *A. calcoaceticus* SC 13876 in the presence of $NAD^+$, glucose, and glucose dehydrogenase reduced **44** to the corresponding monohydroxy compounds **47** and **48** [5-(*S*)-hydroxy-5-oxo-6-(benzyloxy)hexanoic acid ethyl ester **47**, and 3-(*R*)-hydroxy-3-oxo-6-(benzyloxy) hexanoic acid ethyl ester **48**]. Simultaneous reduction of both 3-keto and 5-keto

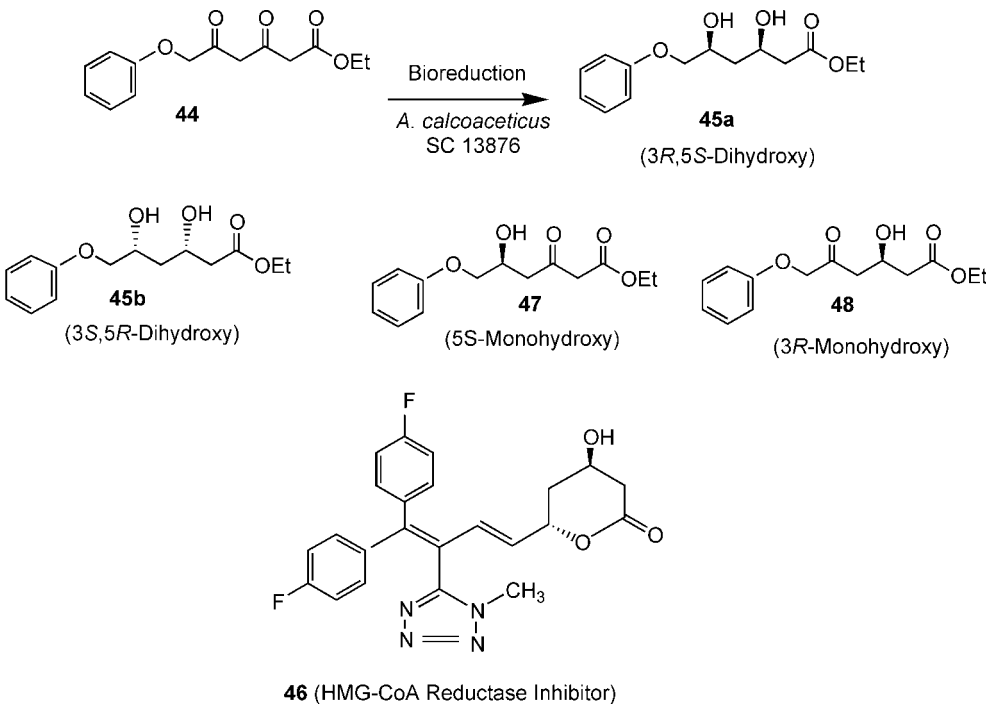


**Figure 14.** Preparation of chiral synthons for the anticholesterol drug **46**: enantioselectiveselective microbial reduction of 3,5-dioxo-6-(benzyloxy)hexanoic acid ethyl ester (**44**).

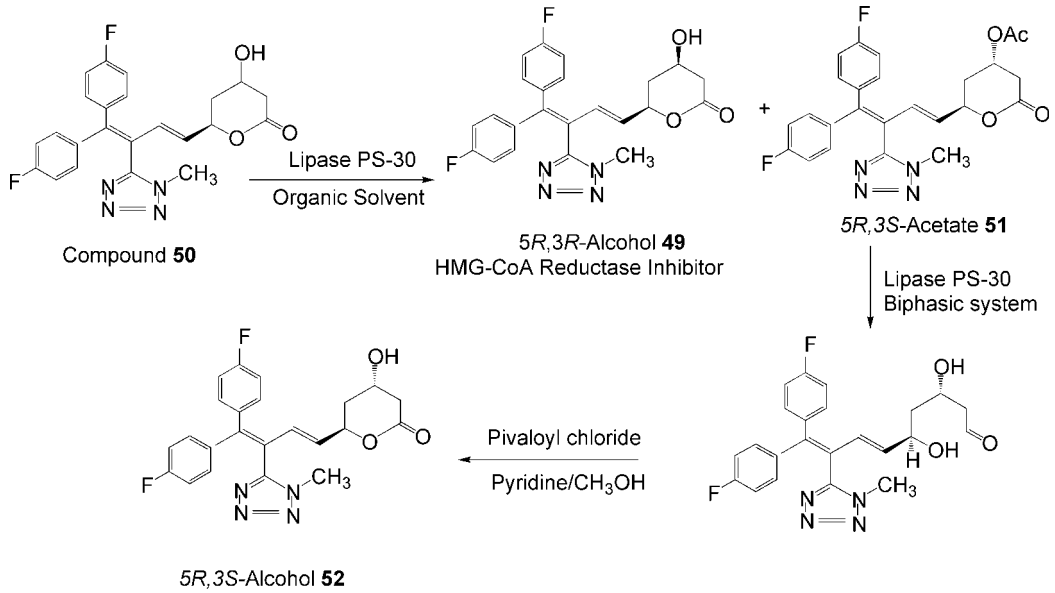


**Figure 15.** Preparation of chiral synthons for the anticholesterol drug **49**: enantioselective enzymatic acetylation of compound **50** to yield (5*R*,3*S*)-acetate **51** and unreacted (5*R*,3*R*)-alcohol **49**.

groups of compound **44** was observed. Both **47** and **48** were further reduced to the (3*R*,5*S*)-dihydroxy compound **45a** by the cell extracts. A reaction yield of 92% and the ee of 98% were obtained when the reaction was carried out in a 1-L batch using cell extracts. The substrate was used at 10 g/L. Product **45a** was isolated from the reaction mixture in 72% overall yield with HPLC purity of 99% and the ee of 98.5%. The reductase from cell extracts of *A. calcoaceticus* SC 13876 was purified about 200-fold. The purified enzyme gave a single protein band on SDS-PAGE corresponding to 33,000 daltons.

Using an enzymatic resolution process, (3*R*,5*R*)-alcohol **49** was also prepared by the lipase-catalyzed enantioselective acetylation of **50** in organic solvent.[81] We evaluated various lipases, among which lipase PS-30 and BMS lipase (produced by fermentation of *Pseudomonas* strain SC 13856) efficiently catalyzed the acetylation of the undesired enantiomer of **50** to yield (5*R*,3*S*)-acetate **51** and unreacted desired (5*R*,3*R*)-alcohol **49** (Figure 15). A reaction yield of 49 M % (theoretical maximum 50 M %) and ee of 98.5% were obtained for (5*R*,3*R*)-alcohol **49** when the reaction was conducted in toluene in the presence of isopropenyl acetate as an acyl donor. The substrate was used at 4 g/L concentration. In methyl ethyl ketone at 50 g/L substrate concentration, a reaction yield of 46 M % and ee of 96% were obtained. The enzymatic process was scaled-up to a 640-L preparative batch using immobilized lipase PS-30 at 3 g/L of substrate **50** in toluene as a solvent. From the reaction mixture product (5*R*,3*R*)-alcohol **49** was isolated in overall 35 M % yield (theoretical maximum yield is 50%) with 98.5% ee and 99.5% chemical purity. The undesired (5*R*,3*S*)-acetate **51** produced by this process was enzymatically hydrolyzed by lipase PS-30 in a biphasic system to prepare the corresponding (5*R*,3*S*)-alcohol **52**. Thus both enantiomers were produced by the enzymatic process.

## 9 Microbial Resolution

### 9.1 Stereoselective Hydrolysis of (*R,S*)-1-(2',3'-Dihydrobenzo[*b*]furan-4'-yl)-1,2-oxirane

Epoxide hydrolase catalyzes the stereoselective hydrolysis of a racemic epoxide to the corresponding chiral diol and unreacted chiral epoxide. Furstoss and his coworkers used *Aspergillus niger* and *Beauveria sulfurescens* for the enantiospecific hydrolysis of epoxides including many substituted styrene epoxides[82–85]; and Faber and coworkers utilized epoxide hydrolases from *Rhodococcus*, *Nocardia*, and other species.[86–90] Enantioselective epoxide hydrolases from various fungal and other sources have been reported.[91,92] Weijers[93] found the yeast *Rhodotorula glutinis* to be effective for enantioselective hydrolysis of various epoxides.

(*S*)-Epoxide **53** is a key intermediate in the synthesis of a number of prospective drug candidates.[94,95] The stereospecific hydrolysis of the racemic epoxide (*R,S*)-1-(2',3'-dihydrobenzo[*b*]furan-4'-yl}-1,2-oxirane (**54**) to the corresponding (*R*)-diol **55** and unreacted (*S*)-epoxide **53** (Figure 16) was demonstrated by Goswami et al.[96]

The chemical stability of the racemic epoxide **54** in aqueous systems under various conditions was deter-

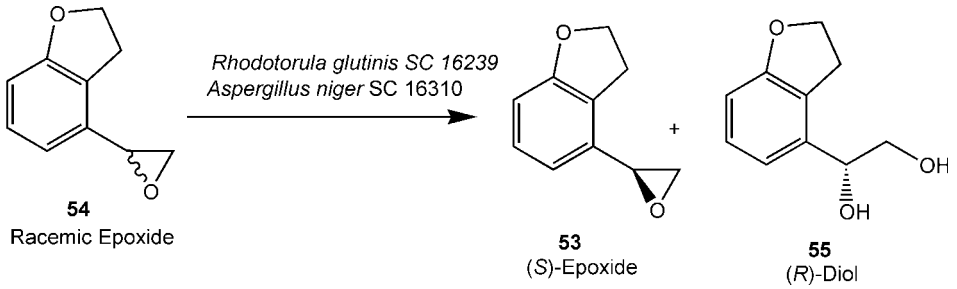


**Figure 16.** Enantioselective hydrolysis of racemic epoxide **54** to the corresponding (*R*)-diol **55** and unreacted (*S*)-epoxide **53**.

mined. At pH 5 and 6, about 100% and 91% of epoxide was hydrolyzed in 24 hours, respectively. Even under neutral conditions (pH 7), the epoxide was not very stable and 51% was hydrolyzed in 24 hours. Alkaline conditions (pH >7) are better and there was less hydrolysis, for example, 38% and 30% in 24 hours at pH 8 and 9, respectively. Therefore, pH 8.0 was selected for conducting the enzymatic hydrolysis. Even at pH 8, 19% of racemic epoxide **54** was hydrolyzed in 4 hours. Therefore, it was necessary to find a microorganism that hydrolyzes the racemic epoxide with high stereospecificity at a faster rate to prevent (or at least minimize) the loss of unreacted desired (*S*)-epoxide **53** by chemical hydrolysis.

Fungal, yeast, and bacterial cultures were screened for hydrolysis of the racemic epoxide. Two *A. niger* strains (SC 16310, SC 16311) and *Rhodotorula glutinis* SC 16293 selectively hydrolyzed the (*R*)-epoxide, leaving behind the (*S*)-epoxide **53,** in >95% ee and 45% yield (theoretical maximum yield is 50%). The enantiomer ratio (E) values for these microorganisms were ~25.

Several solvents at 10% vol/vol were evaluated in an attempts to improve the ee and yield. Solvents had significant effects on both the extent of hydrolysis and the ee of unreacted (*S*)-epoxide **53**. Most solvents [except methyl *tert*-butyl ether (MTBE)] gave a lower ee product and slower reaction rate than that of reactions without any solvent supplement. MTBE gave excellent results. A reaction yield of 45% (theoretical maximum yield is 50%) and an ee of 99.9% were obtained for unreacted (*S*)-epoxide **53**. The hydrolysis reaction in the presence of MTBE gave an E value of 68.

Two *A. niger* strains (SC 16310 and SC 16311) were evaluated for their potential for the enantiospecific hydrolysis of the racemic epoxide. Both strains gave ee (97%) and yield (45%) of the remaining (*S*)-epoxide **53** when substrate was used at 2 g/L concentration. At a higher substrate concentration (5 g/L) using 100 g/L cell concentration, a reaction yield of 51% and ee of 84% were obtained with SC 16311.

### 9.2 Biocatalytic Dynamic Resolution (*R,S*)-1-(2',3'-dihydrobenzo[*b*]furan-4'-yl)-ethane-1,2-diol

One of the most used techniques for the resolution of racemic compounds involves biocatalysis. Although these kinetic resolution processes often provide com-

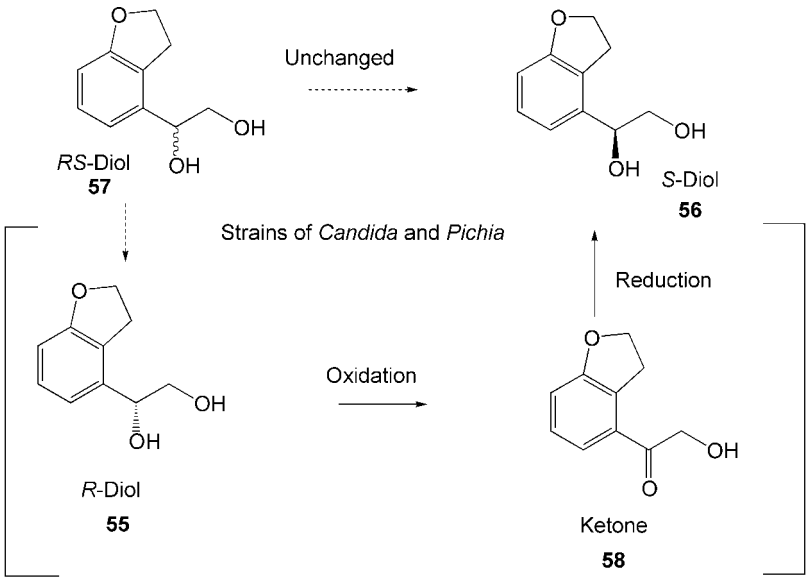


**Figure 17.** Stereoinversion of racemic diol **57** to *S*-diol **56** by *Candida boidinii* and *Pichia methanolica*.

pounds with high ee, the maximum theoretical yield of product or substrate is only 50%. Also, in many cases, the reaction mixture contains a 50:50 mixture of reactant and product with only slight differences in physical properties (e.g., a hydrophobic alcohol and its acetate), and the separation may be very difficult.

These issues with kinetic resolutions can be addressed by employing a "dynamic kinetic resolution" process. A dynamic resolution process for, e.g., resolution of an alcohol, could involve oxidation of one enantiomer of the alcohol to the ketone while the other enantiomer of the alcohol remains unchanged. The ketone is not isolated but is reduced to the opposite desired enantiomer of the alcohol. The net result is the conversion of the racemic alcohol to one enantiomer of the alcohol in high yield (up to 100%).

Only a handful of reports have appeared in the recent literature[97–102] on the dynamic resolution of alcohols. *Geotrichum candidum*, *Candida parapsilosis*, and a few other species are reported to be effective in such processes. Dynamic resolution involving a biocatalyst and metal-catalyzed *in situ* racemizations has also been reported.[103,104]

(*S*)-1-(2',3'-Dihydrobenzo[*b*]furan-4'-yl)-ethane-1,2-diol (**56**) is a potential precursor of epoxide **53**[94,95]. The dynamic resolution of the racemic diol, (*R*,*S*)-1-(2',3'-dihydrobenzo[*b*]furan-4'-yl)-ethane-1,2-diol (**57**) to the (*S*)-diol (Figure 17) was demonstrated, as described below.[105]

Seven cultures (*Candida boidinii* SC 13821, SC 13822, SC 16115, *Pichia methanolica* SC 13825, SC 13860, and *Hansenula polymorpha* SC 13895, SC 13896] were found to be promising for dynamic resolution. During biotransformation, the relative proportions of (*S*)-diol **57** increased with time and at the end of one week, the ee of the remaining (*S*)-diol **57** was found to be in the range of 87 – 100% (yield 60 – 75%) with these microorganisms. Only two microorganisms, *Candida parapsilosis* SC 16346 and *Arthrobacter simplex* SC 6379, showed a higher yield of (*R*)-diol **55**.

A new compound was formed during these biotransformations as evidenced by the appearance of a new peak in the HPLC of the reaction mixture. The identity of this compound was established as the hydroxy ketone **58** from an LC-MS peak at mass 178. The starting (*R*,*S*)-diol showed a mass peak at 180 by LC-MS. The area of the HPLC peak for hydroxy ketone **58** first increased with time, reached a maximum, and then decreased, as expected for the proposed dynamic resolution pathway. *C. boidinii* SC 13822, *C. boidinii* SC 16115, and *P. methanolica* SC 13860 transformed the (*R*,*S*)-diol **57** in 3 – 4 days, to (*S*)-diol **56** in a yield of 62 – 71% and ee's of 90 – 100%.

## 10 Anti-Alzheimer Drugs

(*S*)-(+)-2-Pentanol is a key intermediate required in the synthesis of several potential anti-Alzheimer drugs which inhibit β-amyloid peptide release and/or synthesis.[106,107] The enzymatic resolution of racemic 2-pentanol and 2-heptanol by lipase B from *Candida antarctica* has been demonstrated by Patel et al.[108]

Commercially available lipases were screened for the stereoselective acetylation of racemic 2-pentanol in an organic solvent (hexane) in the presence of vinyl acetate as an acyl donor. *C. antarctica* lipase B efficiently catalyzed the enantioselective acetylation of racemic 2-pentanol. Reaction yields of 49% (theoretical maximum yield is 50%) and an ee of 99% were obtained for the (*S*)-(+)-2-pentanol. Preparative-scale acetylation (100 g input) was carried out. At the end of the reaction, 44.5 g of (*S*)-(+)-2-pentanol were estimated by HPLC analysis with an ee of 98%.

Among the acylating agents tested, succinic anhydride was found to be the best choice due to easy re-

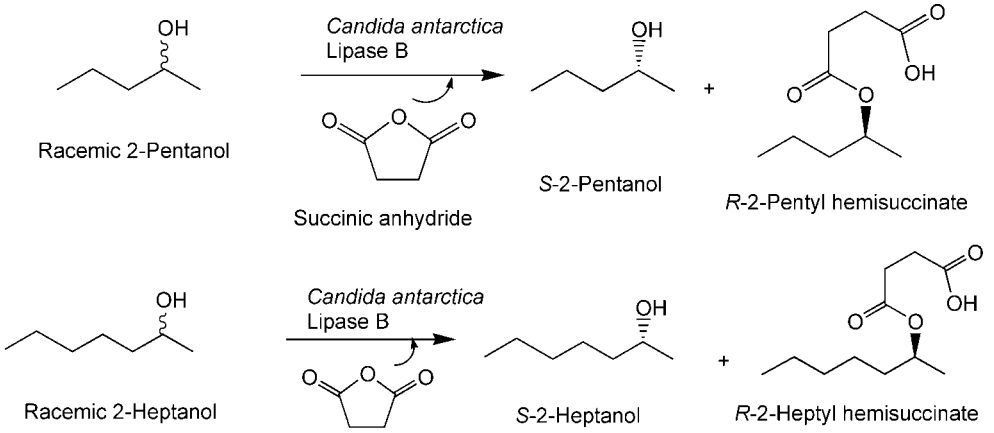


**Figure 18.** Enzymatic resolution of racemic secondary alcohols by *Candida antarctica* lipase.

covery of the (*S*)-2-pentanol at the end of the reaction. Reactions were carried out using racemic 2-pentanol as solvent as well as substrate. Using 0.68 mol-equivalent of succinic anhydride (Figure 18) and 13 g of lipase B per kg of racemic 2-pentanol, a reaction yield of 43 M % (theoretical maximum 50%) and ee of >98% were obtained for (*S*)-(+)-2-pentanol, isolated in overall 38% yield (theoretical maximum 50%). The resolution of 2-heptanol was also carried out using lipase B under similar conditions to give a reaction yield of 44 M % and ee of >99% for (*S*)-(+)-2-heptanol, isolated in 40% overall yield.

## 11 HIV Protease Inhibitor

An essential step in the life cycle of the human immunodeficiency virus (HIV-1) is the proteolytic processing of its precursor proteins. This processing is accomplished by HIV-1 protease, a virally encoded enzyme. Inhibition of HIV-1 protease arrests the replication of HIV *in vitro*. Thus, HIV-1 protease is an attractive target for chemotherapeutic intervention. Recently, Barrish et al[109] reported the discovery of a new class of selective HIV protease inhibitors which incorporates a $C_2$ symmetric aminodiol core as its key structural feature. Members of this class, and particularly compound **59,** BMS-186318, display potent anti-HIV activity in cell cultures. We have described the diastereoselective microbial reduction of (1*S*)-[3-chloro-2-oxo-1-(phenylmethyl)propyl]carbamic acid 1,1-dimethylethyl ester (**60**) (Figure 19) to **61**[110], a key intermediate in the total chemical synthesis of compound **59**.[109]

One hundred microorganisms were screened for the diastereoselective reduction of **60** to **61**. The best four cultures, *Streptomyces nodosus* SC 13149, *Candida boidinni* SC 13821, *Mortierella ramanniana* SC 13850, and *Caldariomyces fumago* SC 13901, gave >39% reaction yields, >91% diastereomeric purities, and 99.9% enantiomeric purities of product **61**.

*Streptomyces nodosus* SC 13149 and *Mortierella ramanniana* SC 13850 were used to convert ketone **60** to the corresponding chiral alcohol **61**. Cells of *Streptomyces nodosus* SC 13149 and *Mortierella ramanniana* SC 13850 were grown in a 25-L fermentor for 48 hours. Cells were collected and suspended in 100 mM potassium phosphate buffer (pH 6.8) and the resulting cell suspensions were used to carry out the two-stage process for biotransformation of **60**. After 24 h, a reaction yield of 67%, an enantiomeric purity of 99.9%, and a diastereomeric purity of >99% were obtained for chiral alcohol **61** using cells of *Streptomyces nodosus* SC 13149. *Mortierella ramanniana* SC 13850 gave a reaction yield of 54%, an enantiomeric purity of 99.9%, and a diastereomeric purity of 90% for chiral alcohol **61**.

A single-stage fermentation-biotransformation process was developed for conversion of ketone **60** to chiral alcohol **61** with cells of *Streptomyces nodosus* SC 13149. A reaction yield of 80%, a diastereomeric purity of >99%, and an enantiomeric purity of 99.8% was obtained. From a 12-L reaction mixture, 6.5 g of chiral alcohol **61** were isolated in 62% overall yield. The diastereomeric purity and the enantiomeric purity of the isolated chiral alcohol were >99% and >99.8%, respectively.

## 12 Conclusion

The production of a single enantiomer of drug intermediates is increasingly important in the pharmaceu-

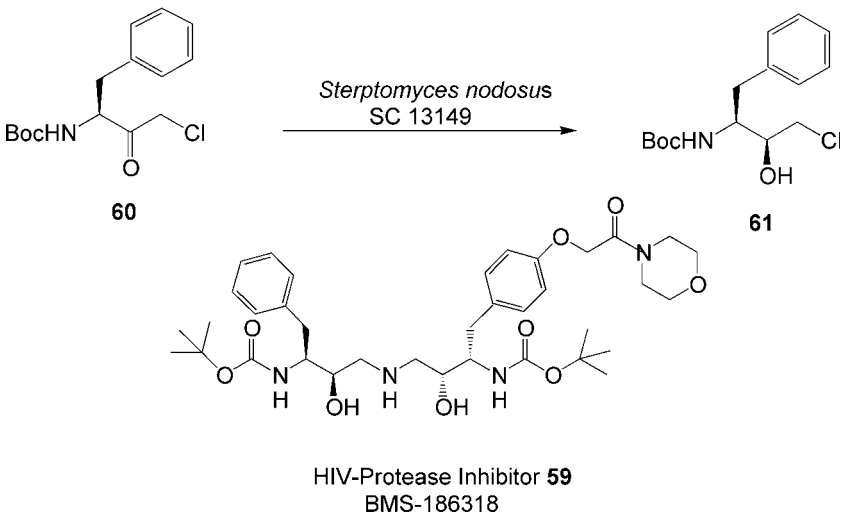


**Figure 19.** Synthesis of chiral intermediates for antiviral agent **59**: diastereoselective enzymatic reduction of (1*S*)-[3-chloro-2-oxo-1(phenylmethyl)propyl]carbamic acid 1,1-dimethylethyl ester (**60**) to the corresponding chiral alcohol **61** by *Streptomyces nodosus* SC 13149.

tical industry. Organic synthesis is one approach to the synthesis of single enantiomers, and biocatalysis provides an added dimension and an enormous opportunity to prepare pharmaceutically useful chiral compounds. The advantages of biocatalysis over chemical catalysis are that enzyme-catalyzed reactions are stereoselective and regioselective and can be carried out at ambient temperature and atmospheric pressure. The use of different classes of enzymes for the catalysis of many different types of chemical reactions is essential to generate a variety of chiral compounds. This includes the use of hydrolytic enzymes such as lipases, esterases, proteases, dehalogenases, acylases, amidases, nitrilases, lyases, epoxide hydrolases, decarboxylases, and hydantoinases in the resolution of racemic compounds and in the asymmetric synthesis of optically active compounds. Oxido-reductases and aminotransferases have been used in the synthesis of chiral alcohols, amino alcohols, amino acids, and amines. Aldolases and decarboxylases have been effectively used in asymmetric synthesis by aldol condensation and acyloin condensation reactions. Monooxygenases have been used in stereoselective and regioselective hydroxylation and epoxidation reactions and dioxygenases in the chemoenzymatic synthesis of chiral diols.

The idea of designing biocatalysts and tailored-made enzymes by random and site-directed mutagenesis and the preparation of thermostable and pH-stable enzymes can lead to the production of novel, stereoselective biocatalysts. In the course of the last decade, progress in biochemistry, protein chemistry, molecular cloning, random and site-directed mutagenesis, and fermentation technology has opened up unlimited access to a variety of enzymes and microbial cultures as tools in organic synthesis.

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